

METHOD FOR OPTICAL DETECTION OF AN ILLUMINATED SPECIMEN  
IN A PLURALITY OF DETECTION CHANNELS

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CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims priority of provisional application Serial  
No. 60/230,344, filed September 6, 2000 and German Application No. 100 33 179.3,  
filed June 29, 2000, the complete disclosure of which is hereby incorporated by  
10 reference.

BACKGROUND OF THE INVENTION

Field of the Invention

15 The invention relates to laser scanning microscopes and, in particular,  
the use of such lasers in a plurality of detection channels.

In laser scanning microscopes, lasers with different wavelengths are  
used for the illumination and excitation of specimens. The light from the specimen  
likewise has different wavelengths depending on the illumination and on the specimen  
20 itself (fluorescence). The light coming back from the specimen is measured and  
evaluated in the form of images. In an n-channel system, all channels must be  
evaluated in order to determine the channels in which data is actually to be detected.  
In microscope systems there are usually two to four recording channels that are  
evaluated according to images. In order to determine the channels in which light is  
25 detected, the specimen must be scanned, and accordingly also illuminated, n/4 times.  
This process is cumbersome and time-consuming and causes an unnecessary loading  
of the specimen by laser light.

OBJECT AND SUMMARY OF THE INVENTION

30 The primary object of this invention is to overcome problems  
associated with the application of microscopes and, particularly, in laser scanning  
microscopes in multi-channel detection systems. In particular, the invention aims at

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simplifying the known process and avoiding unnecessary loading of the specimen by laser light.

In accordance with the invention, a method for the optical detection of an illuminated specimen in a plurality of detection channels comprises assigning an upper and/or lower limiting value which is adjustable for at least one channel and changing the channel to be detected with respect to its mode of operation when the limiting value is reached

#### BRIEF DESCRIPTION OF THE DRAWING

In the drawing, Figure 1 illustrates a schematic block diagram of the inventive arrangement.

#### DESCRIPTION OF THE PREFERRED EMBODIMENTS

In an n-channel detection system D, n comparators K and triggers (one for each detection channel) whose switching thresholds are adjustable by an analog-to-digital converter are incorporated after amplification V. The threshold is adjusted so as to be above the noise level. The output of the comparators is fed in each instance to a register R which has a shared Clear input C and which can be read by a computer. Before measurement, the register (n-bit length) is reset. When a scanning pass is initiated, the comparators that switch are those which receive at their input a signal above the adjusted threshold. Channels which are arranged, e.g., in a wavelength-dependent manner and which do not send a signal above the switching threshold of the comparator are not relevant for evaluation and can be ignored or considered as a sum signal. When the comparator is constructed as a window comparator and both switching thresholds (upper and lower) are adjustable (e.g., via digital-to-analog converters), signals which are either only inside the thresholds or outside (below or above) the thresholds can be separated and evaluated.

The n channels in which data are expected can already be read off in the n-bit length register after a scan. If the detectors are in a spectral arrangement (the manner of this arrangement is optional), the register immediately sends information about the spectral components of the detected light.

This information is an important component for the correct adjustment of an n-channel system. Using this information, all channels can be adjusted to the

respective wavelengths without making any subjective errors. Autoconfiguration (online) of a system with respect to spectral recordings is possible. Further, there are indications for manual optimum selection (offline).

Figure 1 shows a schematic view of the circuit. The diagram illustrates  
5 light entering an n-channel sensor (for example, an n-channel set of photo multiplier tubes). The outputs of each of the sensors are amplified (V), brought a comparator (K) and each comparator output is brought to register R which can be read by computer as discussed above.

The method according to the invention has the following advantages:  
10 in an n-channel detection system, information is immediately available via the active channels;  
when the n detection channels are arranged with spectral sensitivity, information about the spectral components can be displayed immediately;  
15 if there is a plurality of detection channels as recording channels, the repeated scanning for determining where data are available is dispensed with, i.e., reduced loading of the specimen;  
the system can automatically adjust the configuration (active channels and wavelength-dependent adjustments of filter combinations) when the active channels are known;  
20 the system indicates the channels in which data are expected and the wavelength at which this information allows the operator to dispense with time-consuming specimen recordings  
25 with manual adjustment of the system.

While the foregoing description and drawings represent the present invention, it will be obvious to those skilled in the art that various changes may be made therein without departing from the true spirit and scope of the present invention